

Investigation of the effect of the dual orexin receptor antagonist almorexant on ophthalmological, spermatogenic, and hormonal variables in healthy male subjects

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ABSTRACT

Background/Aims: The aim of this single-center, double-blind study was to investigate the effect of a 4-week once daily administration of 200 mg almorexant on tear film break-up time, spermatogenesis, hormone levels, and pancreatic elastase in stool in healthy male subjects.

Methods: Almorexant 200 mg or matching placebo was administered in the evening for 4 weeks once daily to 56 healthy male subjects. Changes in ophthalmological variables, sperm composition, hormone levels, and pancreatic elastase levels in stool were evaluated periodically up to 8 weeks after discontinuation of drug administration. Blood samples for pharmacokinetic measurements were taken after 4 weeks to confirm compliance to study drug intake.

Results: The results of this study revealed no treatment effects of almorexant, neither on tear film break-up time nor on other ophthalmological variables investigated during this study. Furthermore, spermatogenesis, hormones of the hypothalamic-pituitary-adrenal and -gonadal axes, and endocrine pancreatic secretion were shown to be not affected by a 4-week once daily administration of almorexant.

Conclusion: Almorexant was well tolerated and had no effect on the spectrum of pharmacodynamic variables assessed. Ophthalmology and testicular findings detected in preclinical studies were not observed in this clinical study. Therefore, these preclinical findings appear not to be relevant for humans and do not prevent from conducting larger clinical trials with either healthy subjects or patients.

1. Introduction

The orexin system plays an important role in the regulation of arousal and sleep-wake balance as well as of energy homeostasis and reward and stress processes [1–5]. Two neuropeptides, orexin-A and orexin-B bind to and activate two closely related G protein-coupled receptors, orexin-1 (OX₁) and orexin-2 (OX₂) [5]. The orexin peptides are derived from a single precursor protein (prepro-orexin) and both the peptides and their receptors are highly conserved across vertebrate species. In human brain, orexins are synthesized exclusively by only approximately 70 000 neurons in the perifornical and lateral hypothalamic areas [6,7]. From the lateral hypothalamus orexin-containing neurons widely project into different brain regions such as the locus coeruleus and raphe nucleus that are related to waking and regulation of slow-wave and rapid-eye-movement (REM) sleep [8–10]. It has been shown in several species including man that orexin-A levels are higher during active waking periods and that the concentration of this peptide is decreased in the cerebrospinal fluid of narcoleptic individuals [11, 12]. Orexin receptors may be a therapeutic target for treatment of sleep disorders, obesity, emotional stress, and addiction [5,13–17].

Almorexant was the first dual orexin receptor antagonist (DORA) to be investigated in clinical studies. It decreases alertness and wakefulness in a dose-dependent manner in rats, dogs, and humans [18,19] and blocks both OX₁ and OX₂ receptors with almost equimolar potency with an IC₅₀ of 16 and 15

nM, respectively [18]. In rats almorexant prolonged total sleep time (both non-REM and REM without the disruption of sleep

architecture seen with other hypnotics [18]. A first-in-human study in healthy subjects receiving a single dose of almorexant confirmed the sleep-enabling effects in humans. Vigilance, alertness, and visuomotor coordination were dose-dependently reduced following daytime administration of almorexant at doses of ≥ 400 mg [19]. Similar findings were obtained during administration of almorexant to healthy adult subjects for up to 6 days and at doses up to 1000 mg once daily [20]. No tolerance or loss of efficacy developed over time with repeated dosing. The clinical utility of DORAs in the treatment of insomnia was first demonstrated in a proof-of-concept study with almorexant [14]. The lowest effective dose following evening administration to patients was 100 mg.

Thus far two DORAs have reached the market, i.e., suvorexant and lemborexant. Clinical development of almorexant as an oral therapy was discontinued during Phase 3 due to infrequent clinical laboratory abnormalities in a drug-drug interaction study [21] but the compound is still under exploration for other routes of administration and is used as a pharmacological tool to perform mechanistic studies [22]. In preclinical chronic toxicology studies high doses of almorexant showed no effects on the physiological function of the cardiovascular, respiratory or neuromuscular systems. In general, dose-related toxicity was found in testes, epididymides, prostate, and adrenals in

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rats and dogs as well as in pancreas, gastrointestinal tract, meibomian and sebaceous glands, and skin in dogs. These findings were all reversible after 4 weeks of recovery. Based on the no-observed-adverse-effect-level in dogs during the 13-week toxicity study and the mean exposure in humans at the 200 mg dose level, the calculated safety margin for meibomian gland changes in humans was approximately 1. Rats did not show any ophthalmological findings whereas examination in the dog revealed epiphora and kerato-conjunctivitis sicca. Microscopically, atrophy in the meibomian gland was noted. The effects observed in the testes of both species are significantly reduced total sperm counts and degeneration of elongating spermatids, characterized by an altered shape and a condensation of the developing spermatid head. The findings were restricted to late spermiogenesis as there were no other histological effects on Sertoli cells, Leydig cells, spermatogonia, spermatocytes or round spermatids after 4 and 13 weeks of treatment.

Before exposing a large population for weeks or months to almorexant in clinical trials, the objectives of this study were to investigate the tolerability of a 4-week administration of almorexant to healthy subjects with a special emphasis on the target organs of preclinical toxicity. The primary objective was to demonstrate that a 4-week once daily treatment with almorexant 200 mg did not have a clinically relevant effect on meibomian gland function (assessed by measuring the tear film break-up time [TFBUT]), whereas secondary objectives included tear film quality, semen analysis, plasma concentrations of hormones of the hypothalamic-pituitary-adrenal and -gonadal axes, and pancreatic elastase concentration in stool. A battery of hormones was assessed because of significant evidence that orexins play an important role in driving both behavioral and neuroendocrine responses to challenges that evoke hypothalamic-pituitary-adrenal axis activation [23,24].

2. Methods

2.1. Subjects

Healthy male subjects aged between 18 and 45 years and a body mass index (BMI) of 18–28 kg/m² were enrolled in the study if they were of good health as assessed by physical examination and standard laboratory tests. Subjects with excessive caffeine consumption, a history of alcoholism or drug abuse as well as those wearing contact lenses and those treated with hormones and/or hormone-suppressing agents or retinoids within 6 months prior to screening were not eligible to participate in the study. Subjects had to meet the following criteria ensuring the absence of signs or symptoms of dry eyes, i.e., ocular surface disease index (OSDI®) < 15, TFBUT > 11 s, total ocular surface staining grade (TOSSG) < 5, Schirmer test > 5.5 mm (after 5 min), and normal meibomian gland function (i.e., meibomian gland dysfunction scale [MGDS] aggregate score ≤ 2). In addition, subjects' semen had to fulfill all following criteria: concentration ≥ 20 × 10⁶/mL, motility ≥ 25 % (i.e., ≥ 25 % of sperm with fast/rapid or slow progressive motility), and strict normal morphology ≥ 5 % (i.e., ≥ 5 % of sperm

meeting strict normal morphology criteria [25]). No concomitant medication was allowed during the study except for the treatment of adverse events (AEs). All subjects gave written informed consent. The study was conducted in accordance with good clinical practice (GCP) and the Declaration of Helsinki at a single center in Bloemfontein, South Africa. The study was approved by the Ethics Committee of the Faculty of Health Sciences, University of the Free State, Bloemfontein, Republic of South Africa.

2.2. Study design

This was a prospective, single-center, randomized, double-blind, placebo-controlled, parallel-group, multiple-dose, Phase 1 study in which the non-inferiority of 200 mg almorexant administered for 4 weeks versus placebo was investigated in 56 healthy male subjects. The study consisted of a 4- to 14-day screening period including an adaptation night during which baseline values were recorded, a 4-week treatment period, a follow-up period of ≥ 8 weeks, and a 4-week conditional recovery period. During the treatment period study drug was administered in the clinic in the evening approximately 3 h after the subjects received an evening meal and prior to going to bed. Almorexant was provided as capsules of 100 mg, i.e., each evening 2 capsules were administered to subjects on active drug. Those subjects assigned to placebo received 2 matching placebo capsules that were identical to those of almorexant except for the active ingredient. The subjects stayed in the study center overnight and were allowed to leave in the next morning only if they felt well and did not experience any symptoms possibly related to sleep-promoting medication. A urine drug screen and various study assessments were performed on a weekly basis. An ophthalmologic examination was conducted at baseline (adaptation night), weeks 2, 4, and 12. Semen analysis was performed at screening, week 4, and week 12. If the latter analysis revealed abnormal results, another test was performed at week 16 after an additional 4-week recovery period. A feces sample was collected at baseline and after 4 weeks of treatment. In addition, a blood sample was collected at baseline and after 4 weeks of treatment to determine hormone and almorexant plasma concentrations.

2.3. Safety and tolerability

Safety and tolerability assessments included clinical laboratory tests, urinalysis, vital signs, 12-lead electrocardiogram (ECG) examination, and the monitoring of AEs. Additionally, the narcoleptic effects questionnaire [26] and the Epworth Sleepiness Scale (ESS) [27] were used to detect symptoms of cataplexy and evaluate daytime sleepiness, respectively.

2.4. Pharmacokinetics

Blood samples (4 mL) for the determination of almorexant were collected within 3 h of awakening in the morning (approximately 11 h post-dose) after the last day of dosing. The samples were collected in tubes containing EDTA, immediately put on ice, and centrifuged for 10 min at approximately 2000 × g and 4 °C. After processing, samples were frozen and

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kept at -20°C until analysis. Concentrations of almorexant in plasma were determined with the use of a validated chromatography coupled to tandem mass spectrometry (LC-MS/MS) assay with a lower limit of quantification (LOQ) of 0.5 ng/mL [19]. The interassay accuracy for this method was between 105.5 and 113.3 % and the interassay precision was $<7.1\%$.

2.5. Pharmacodynamics

2.5.1. Ophthalmological examination

Ophthalmological examinations were performed at baseline, week 2, week 4, and week 12 on both eyes using assessments of TFBUT, the subject-recorded questionnaire OSDI, TOSSG, Schirmer test, and MGDS. TFBUT, TOSSG, and Schirmer test were conducted as described previously [28] whereas OSDI was performed as described by Schiffman et al. [29]. An ophthalmological examination was abnormal if 3 or more of the following criteria were met: TFBUT ≤ 10 s, OSDI ≥ 15 , TOSSG ≥ 5 , Schirmer test ≤ 5.5 mm (after 5 min), or MGDS > 2 .

2.5.2. Semen analysis

Semen sampling was scheduled at baseline, week 4, the week-12 follow-up, and, if applicable, the week 16 recovery visit. Semen samples were kept between 20 and 40 $^{\circ}\text{C}$ until analysis. All semen samples were evaluated macroscopically and microscopically within 1 h after collection according to the procedures described in the World Health Organizations' laboratory manual for semen analysis [25]. A semen sample was considered abnormal if one or more of the following criteria regarding spermatozoa were met: concentration $<10 \times 10^6/\text{mL}$, $<25\%$ of sperm with fast/rapid or slow progressive motility, or $<5\%$ of sperm with strict normal morphology.

2.5.3. Hormones

Blood samples (20 mL) for hormone measurements were collected at baseline and week 4 in 3 tubes; 1 containing EDTA and 2 without anticoagulant. EDTA-containing samples were immediately cooled on ice. All blood samples were centrifuged for 10 min at approximately $2000 \times g$ and $2-8^{\circ}\text{C}$ and stored at -20°C until they were analyzed using standard commercially available assay kits. Adrenocorticotrophic hormone (ACTH), cortisol, dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT), follicle-stimulating hormone (FSH), ghrelin, leptin, luteinizing hormone (LH), prolactin, testosterone, and thyroid-stimulating hormone (TSH) were measured in plasma. Inhibin B, triiodothyronine (T3), and thyroxine (T4) were determined in serum.

2.5.4. Pancreatic elastase

Feces samples were collected within 24 h before the adaptation night (baseline) and at the end of treatment visit (week 4 or premature discontinuation). The amount of pancreatic elastase in feces as a measure of exocrine pancreatic secretion was determined by an enzyme-linked immunosorbent assay (ELISA) specified for human pancreatic elastase with a measurement range of 0.015 to 0.500 mg elastase 1 per gram feces.

2.6. Statistics

2.6.1. Statistical hypotheses

The null hypothesis in this non-inferiority study was that the incidence of the primary endpoint (TFBUT ≤ 10 s at week 4) in the active treatment group exceeds by 25 % the incidence in the placebo group. The alternative to be detected was that the incidences are equal in the treatment groups. TFBUT was expected to be normally distributed with a mean value of 18 s and a standard deviation of 5 s; the incidence of TFBUT ≤ 10 s in subjects on placebo at week 4 was therefore expected to be approximately 5%. An absolute increase in incidence of 25 % was considered to be the minimum increase of clinical relevance.

2.6.2. Statistical analysis

The non-inferiority null hypothesis (primary endpoint) was tested by computing the upper limit of the exact 97.5 % confidence limit (CL) of the difference of proportions and comparing it to the 25 % threshold. The sample size was chosen such that the null hypothesis could be answered with a power of 91 % under the assumption that the incidence of the primary endpoint in both the placebo and active treatment group is 0.05, with 25 subjects in each group. Secondary and explanatory endpoints as well as PK parameters and safety data were summarized descriptively. SAS software, version 8.2 (SAS institute, Cary, NC, USA) was used for the statistical analysis and reporting of the clinical data. PROC BINOMIAL was used to perform the unconditional test of non-inferiority of the primary endpoint.

3. Results

3.1. Demographics and baseline characteristics

A total of 56 healthy male subjects aged 22.4 ± 3.8 years (mean $\pm$ SD) with a mean body weight of 77.3 ± 9.1 kg and a BMI of 23.8 ± 2.2 kg/m² participated in this study. All 56 subjects in the study were Caucasian. At baseline (adaptation night), values for ophthalmological assessments, semen analysis, ESS responses, vital signs, and body weight were comparable between the two treatment groups. All but two subjects who withdrew consent completed the treatment period. Another 4

subjects were lost to follow-up, i.e., did not complete the week 12 or week 16 visit.

3.2. Safety and tolerability

Almorexant administration for 4 weeks was well tolerated. Treatment-emergent AEs were reported by 15 subjects during this study. Of those, 9 subjects (32.1 %) received placebo and 6 (21.4 %) almorexant. The most frequently reported treatment-emergent AEs that occurred during the conduct of this study were nasal congestion, headache, and myalgia (Table 1). All AEs were of mild or moderate intensity and resolved without sequelae. One subject died in a road traffic accident 23 days after the end of almorexant treatment. The evaluation of clinical laboratory variables revealed 2 subjects in the almorexant group with an asymptomatic increase in liver aminotransferases ($<3 \times$ the upper limit of normal) at week 4 that normalized within 10 days.

There were no other treatment-emergent abnormalities in laboratory variables, vital signs, body weight, quantitative ECG variables, or occurrence of ECG abnormalities. The responses to the narcoleptic effects' questionnaire or mean scores for ESS between the almorexant and the placebo group were comparable.

3.3. Pharmacokinetics

In this study, pharmacokinetic assessments were only performed to confirm compliance to study drug intake. All 27 subjects in the

Table 1

Summary of most frequently treatment-emergent adverse events.

Adverse event, n (%)	Placebo (n = 28)	Almorexant 200 mg (n = 28)
Subjects with ≥ 1 AE	9 (32.1)	6 (21.4)
Total number of different AEs	11	9
Nasal congestion	4 (14.3)	2 (7.1)
Headache	–	1 (3.6)
ALT increased*	–	1 (3.6)
AST increased*	–	1 (3.6)
Fatigue	–	1 (3.6)
Hematuria	–	1 (3.6)
Musculoskeletal stiffness	–	1 (3.6)
Somnolence	–	1 (3.6)
Myalgia	2 (7.1)	–
Arthralgia	1 (3.6)	–
Dyspepsia	1 (3.6)	–
Eye irritation	1 (3.6)	–
Fecaloma	1 (3.6)	–
Removal of foreign body from eye	1 (3.6)	–

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

* Below 3x upper limit of normal.

almorexant group that completed the 4-week treatment period had measurable almorexant plasma concentrations indicating a good compliance. The mean plasma concentration measured 3 h after awakening (approximately 11 h after drug administration) was $17.1 \text{ ng/mL} \pm 13.2$ (mean $\pm$ SD).

3.4. Pharmacodynamics

3.4.1. Primary endpoint (TFBUT ≤ 10 s at Week 4)

The administration of 200 mg almorexant once daily for 4 weeks showed no increase in the incidence of TFBUT ≤ 10 s compared to placebo in the 54 subjects (27 on almorexant, 27 on placebo) that completed the treatment period. TFBUT ≤ 10 s after 4 weeks of treatment was observed in 8 subjects on placebo (29.6 %) but in only 5 subjects on almorexant (18.5 %) indicating that almorexant was not inferior to placebo (Table 2). Therefore, the null hypothesis was rejected (97.5 % upper treatment difference CL = 0.1358, $P < 0.0001$).

3.4.2. Secondary endpoints

3.4.2.1. Tear film break-up time. As in the analysis of the primary endpoint, administration of almorexant did not lead to an increase in the incidence of TFBUT ≤ 10 s at week 2 and week 12. The incidence of

TFBUT ≤ 10 s at week 2 was the same in the almorexant compared to the placebo group. At week 12 the incidence of TFBUT ≤ 10 s was higher than observed at weeks 2 and 4. However, the incidence was comparable between the two treatment groups. Thus, the incidence of TFBUT ≤ 10 s in the almorexant group was at no time point higher than in the placebo group supporting the rejection of the null hypothesis (Fig. 1).

3.4.2.2. Ophthalmological assessments. The other ophthalmological assessments in this study included the OSDI, total ocular surface staining grade, Schirmer test, and MGD scale and were conducted at the adaptation night (baseline), weeks 2, 4, and 12. The changes from baseline to these time points are summarized in Table 3.

No statistically significant differences between the treatment groups could be discerned in any of the ophthalmological assessments as indicated by the mean treatment effects and their 95 % CL as well as by the change in absolute values over time (Fig. 2) which only show small mean changes in both treatment groups over the 12 weeks, except for the Schirmer test. This test showed a mean increase in reflex tear flow over time in the almorexant group, whereas a decrease was observed in the placebo group, resulting in mean treatment effects (95 % CL) of 4.9 mm (0.0, 9.8), 5.3 mm (1.0, 9.7), and 7.8 mm (3.3, 12.3) at weeks 2, 4, and 12, respectively.

None of the subjects receiving almorexant met prespecified criteria for abnormal ophthalmological examination at any time point.

3.4.2.3. Semen analyses. The baseline values for the sperm concentration, morphology, and motility measured during the adaptation night were comparable between the two treatment groups. After 4 weeks of study drug intake, the mean concentration of spermatozoa decreased,

Table 2

Incidence of tear film break-up time (TFBUT) ≤ 10 s at Week 4.

	Placebo (n = 27) ^a	Almorexant 200 mg (n = 27) ^a
Subjects with BUT ≤ 10 s, n (%)	8 (29.6)	5 (18.5)
95 % CI	13.8 - 50.2	6.3 - 38.1
Treatment effect:		
p-value	<0.0001	
97.5 % upper CL for treatment difference	0.1358	

^a Two subjects, one in each treatment group, withdrew consent and did not complete the 28-day treatment period. CI, confidence interval; CL, confident limit.

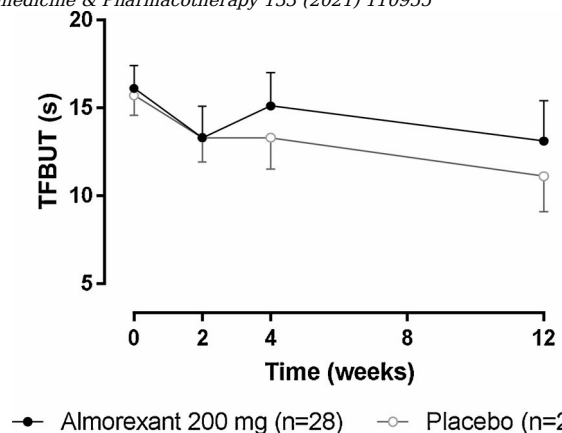


Fig. 1. Time course of mean (95 % CI) tear film break-up time. CI = confidence interval; TFBUT = tear film break-up time.

whereas the mean proportion of spermatozoa with normal morphology increased in the almorexant as well as in the placebo group (Fig. 3). The difference between the treatment groups was negligible. The mean changes in the proportion of spermatozoa with fast/rapid or slow progressive motility measured at week 4 showed a decrease in the almorexant group (-1.6 %) and an increase in the placebo group (+4.6 %). However, the mean treatment effect (-6.2 %, 96 % CL -13.7, 1.3) showed no statistically significant difference between the two treatment groups.

The incidence of an abnormal semen analysis at weeks 2, 12, and 16 was an additional marker based on which the effect of almorexant on human semen was investigated during this study. At baseline, all subjects had normal semen analyses. At week 4, 2 subjects in each treatment group on almorexant and placebo met the criteria for an abnormal semen analysis as did 3 (11.5 %) and 5 (17.9 %) subjects, respectively, at week 12. All subjects that completed the additional follow-up at week 16 had a normal semen analysis indicating that no irreversible effect on semen quality and quantity could be observed.

Table 3

Ophthalmologic assessments: Changes from baseline to Week 2, Week 4, and Week 12 comparing placebo and Almorexant 200 mg.

Ophthalmologic assessments	Placebo mean (SD)	Almorexant 200 mg mean (SD)
<i>Ocular surface disease index (OSDI)</i>		
Change from baseline to Week 2	- 0.7 (4.2)	0.8 (3.6)
Change from baseline to Week 4	- 1.4 (4.6)	- 0.3 (2.7)
Change from baseline to Week 12	- 0.4 (4.5)	- 0.2 (3.1)
<i>Tear break-up time (s)</i>		
Change from baseline to Week 2	- 2.4 (4.5)	- 2.8 (5.1)
Change from baseline to Week 4	- 2.4 (5.5)	- 1.0 (6.4)
Change from baseline to Week 12	- 4.6 (5.3)	- 3.1 (6.7)
<i>Ocular surface staining grade (TOSSG)</i>		
Change from baseline to Week 2	0.4 (0.7)	0.1 (0.7)
Change from baseline to Week 4	0.4 (0.6)	0.1 (0.5)

Change from baseline to Week 12	0.4 (0.6)	0.3 (0.8)
<i>Schirmer test-worst value (mm)</i>		
Change from baseline to Week 2	- 2.2 (6.8)	2.7 (10.7)
Change from baseline to Week 4	- 2.4 (5.3)	2.9 (10.1)
Change from baseline to Week 12	- 5.7 (7.7)	2- 1 (8.5)
<i>Meibomian gland dysfunction scale</i>		
Change from baseline to Week 2	0.8 (1.4)	0.6 (1.3)
Change from baseline to Week 4	0.7 (1.5)	0.8 (1.7)
Change from baseline to Week 12	0.4 (1.5)	0.6 (1.5)

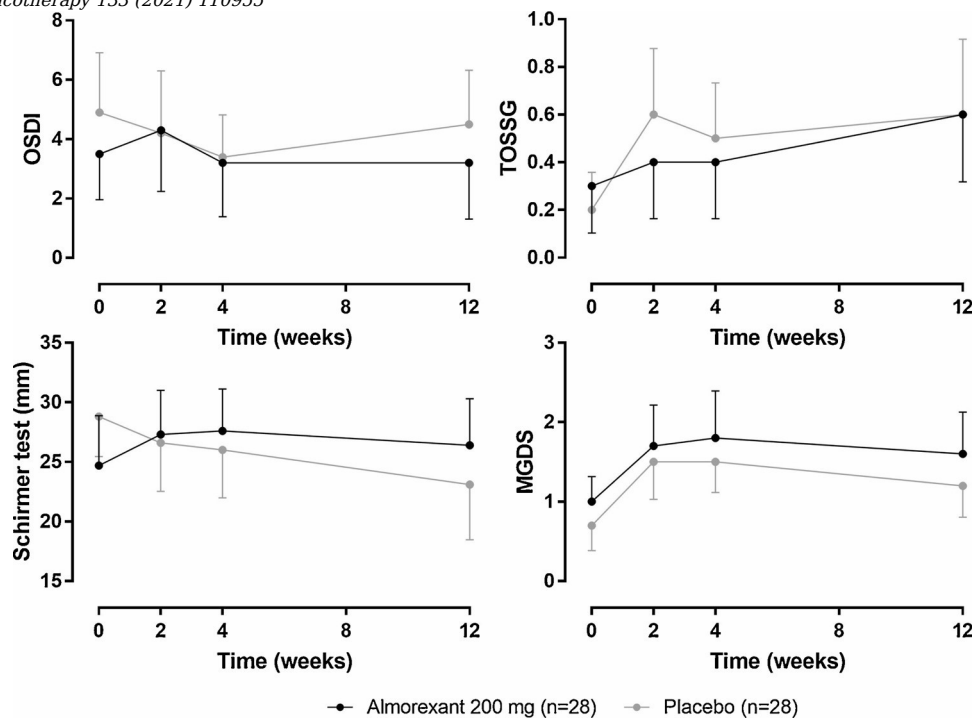


Fig. 2. Time course of mean (95 % CI) values of OSDI, TOSSG, Schirmer test, and MGDS. CI = Confidence interval; MGDS = Meibomian Gland Dysfunction Scale; OSDI = Ocular Surface Disease Index; TOSSG = Total Ocular Surface Staining Grade.

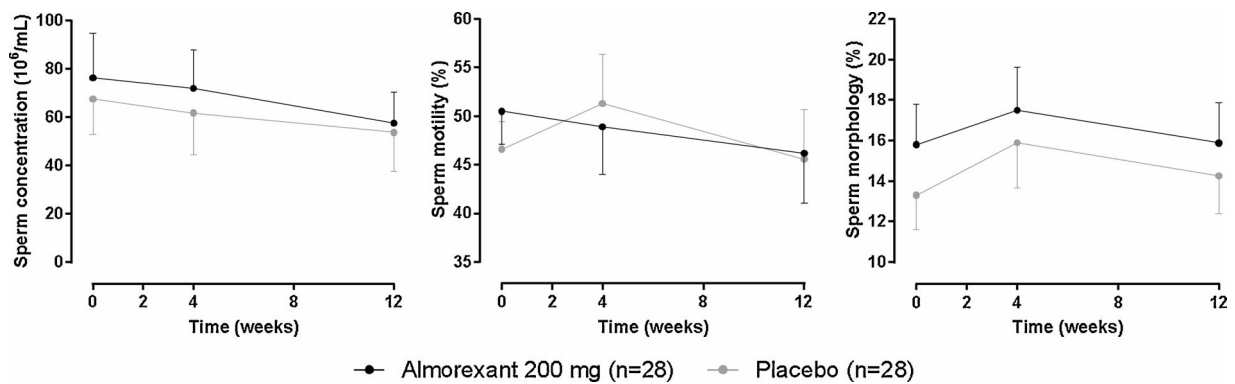


Fig. 3. Time course of mean (95 % CI) values of sperm concentration, motility, and morphology. CI = Confidence interval.

3.4.2.4. Plasma and serum hormone concentration.

Mean concentrations of all hormones measured at baseline and week 4 are presented in Fig. 4. The mean plasma concentration of testosterone was higher in the almorexant compared to the placebo group at both baseline and week 4. After 4 weeks of study drug administration, the testosterone concentration decreased in the almorexant group, whereas an increase was observed in the placebo group, resulting in a mean treatment effect of -17 ng/mL (95 % CL -33 , -1.9). However, the observed effect was not considered as clinically relevant due to the fact that the mean and minimum levels were similar between the treatment groups at week 4. Furthermore, DHT, the active metabolite of testosterone, was not shown to be decreased at week 4. Similar to testosterone, a mean decrease in ACTH concentrations was observed at week 4 in the almorexant group with a mean treatment effect of -14.1 pg/mL (95 % CL -27 , -1.2). However, the concentration was still well within the normal range (10–60 pg/mL between 8:00–10:00). The individual data points for testosterone and ACTH in both

treatment groups are presented in Fig. 5. For the other hormones, no significant differences could be detected between the two treatments.

3.4.2.5. Pancreatic elastase. Almorexant had no effect on the levels of pancreatic elastase in stool. Only one subject had a value ≤ 0.2 mg/g (0.121 mg/g at baseline) during the study.

4. Discussion

Orexin-containing neurons spread widely across the central nervous system and, therefore, antagonism of orexin receptors could theoretically lead to a spectrum of different effects, including those on hormonal systems [30]. The DORA almorexant had, until the conduct of the present study, been investigated in a series of clinical studies in healthy subjects and insomnia patients with a maximum treatment duration of up to 16 days [31,32]. Before initiating long-term studies in patients with insomnia disorders, it was considered worthwhile to first explore in more detail several findings observed in toxicology studies in rats and dogs. Based on the nature of these findings they could not be unequivocally attributed to the drug's mechanism of action and mere compound- or species-specific findings could not be excluded.

Treatment with almorexant 200 mg every evening for 4 weeks was well tolerated in this study. Few AEs were reported and their pattern as well as the drug's plasma concentrations were in accordance with previous findings [20,31]. In particular, there were no signs of next-day residual drowsiness. The small increases in liver aminotransferases in 2 subjects are not uncommon in studies with healthy subjects who are treated for 4 weeks on an ambulatory basis [33].

However, this requires monitoring of patients in studies of larger sample size and longer duration to investigate whether this phenomenon is drug related or was a chance finding in the present study. The drug levels measured are like those obtained after single- and multiple-dose administration, in line with the minimal level of accumulation of almorexant [19,20,31]. Tolerance development has not been reported for DORAs, neither from a pharmacokinetic nor from a pharmacodynamic point of view although suvorexant has been on the market since 2014.

The lowest safety margin, approximately 1 in relation to the mean exposure at the clinical therapeutic dose of 200 mg almorexant, was determined for meibomian gland changes. Therefore, the present clinical study was statistically designed as a non-inferiority study comparing the incidence of TFBUT ≤ 10 s between the active

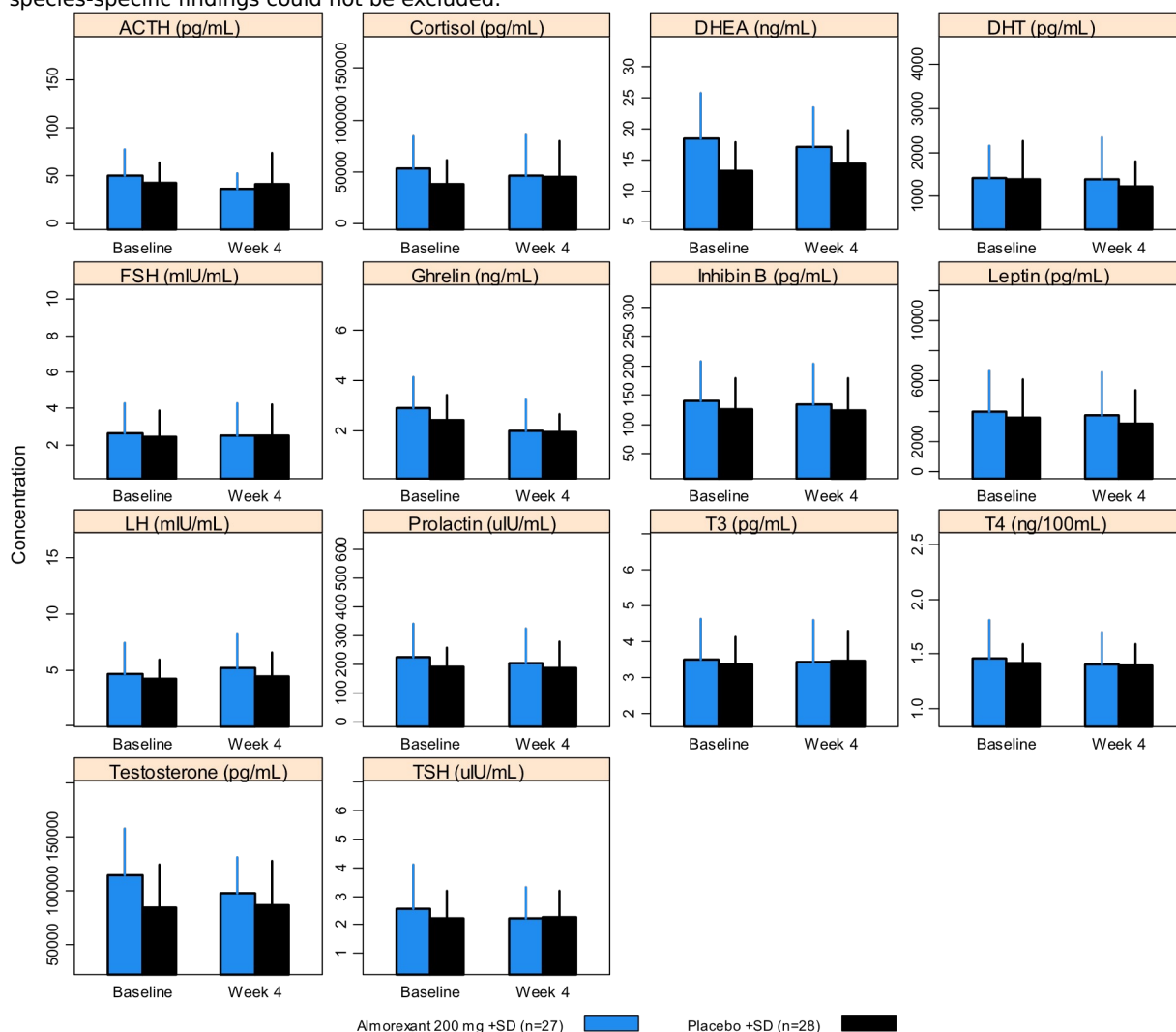


Fig. 4. Mean concentration (95 % CI) of hormones following 4 weeks of once daily administration of 200 mg almorexant or placebo.

treatment and placebo. In addition, to detect clinically significant changes in testicular function would require a considerably larger sample size because of the pronounced intra- and interindividual variability in its variables.

Meibomian gland function is most often investigated in the context of dry eye syndrome and other ocular surface disorders and seldom as a potential adverse reaction to drug treatment [34]. The meibomian glands produce lipids that are the main component of the lipid layer of the tear film that protects it against evaporation of the aqueous phase [35]. In healthy subjects, the tear film is rebuilt with each blink and remains stable during the interblink interval. Most investigations focus on the meibomian glands in the lower lid because of their greater accessibility although the secretory capacity of the glands in the upper lids is approximately double that in the lower lids. In contrast to sebaceous glands in general, there is relatively little information on the neural influence of the meibomian gland [36,37]. As the rate of meibomian lipids delivery is dependent on age, sex, and diurnal conditions [35,38,39], a homogeneous population of healthy young male subjects underwent eye examinations at fixed time points. In particular, the role of androgens in regulating meibomian gland function has been well demonstrated [40]. In view of the highly dynamic characteristics of the tear film, in this study several standard noninvasive assessment procedures used in the diagnosis of tear film disorders were applied with a focus on objective and quantifiable response measures.

The TFBUT as determined with fluorescein assesses the stability of the tear film whereas the Schirmer test measures the lacrimal gland

production capacity [28,39]. The Schirmer test has been widely used in assessing the adequacy of tear production [41,42]. Although the limitations of this test, originally introduced in 1903, are well known, it was applied in this study because it yields information complementary to the other tests. Damage of ocular surface was explored via staining. In addition to the objective tests, subjective symptoms and functional lifestyle were assessed by use of the OSDI, a validated questionnaire, as often the correlation between objective clinical signs and patient symptoms is not strong [29]. The OSDI has some limitations as it is based on visual performance but, due to its extensive use, was included in this study [28,29].

The results of this study demonstrate that a 4-week once-daily treatment with 200 mg almorexant was not inferior to placebo on the incidence of TFBUT ≤ 10 s, the primary endpoint in this study. Additionally, no differences between treatments were observed in any of the secondary ophthalmological variables.

The cycle of human sperm development takes approximately 74 days [43]. As findings in the animal toxicology studies were limited to late spermiogenesis, a treatment duration of 4 weeks in this clinical study was considered enough to detect relevant findings in man. A 4-week treatment period with almorexant 200 mg did not elicit any discernible effect on sperm concentration, motility or morphology.

The extensive hormonal assessments in this study were mainly performed based on the preclinical testicular findings but also because the meibomian gland function is influenced by hormonal abnormalities [44]. In studies with primary cultures

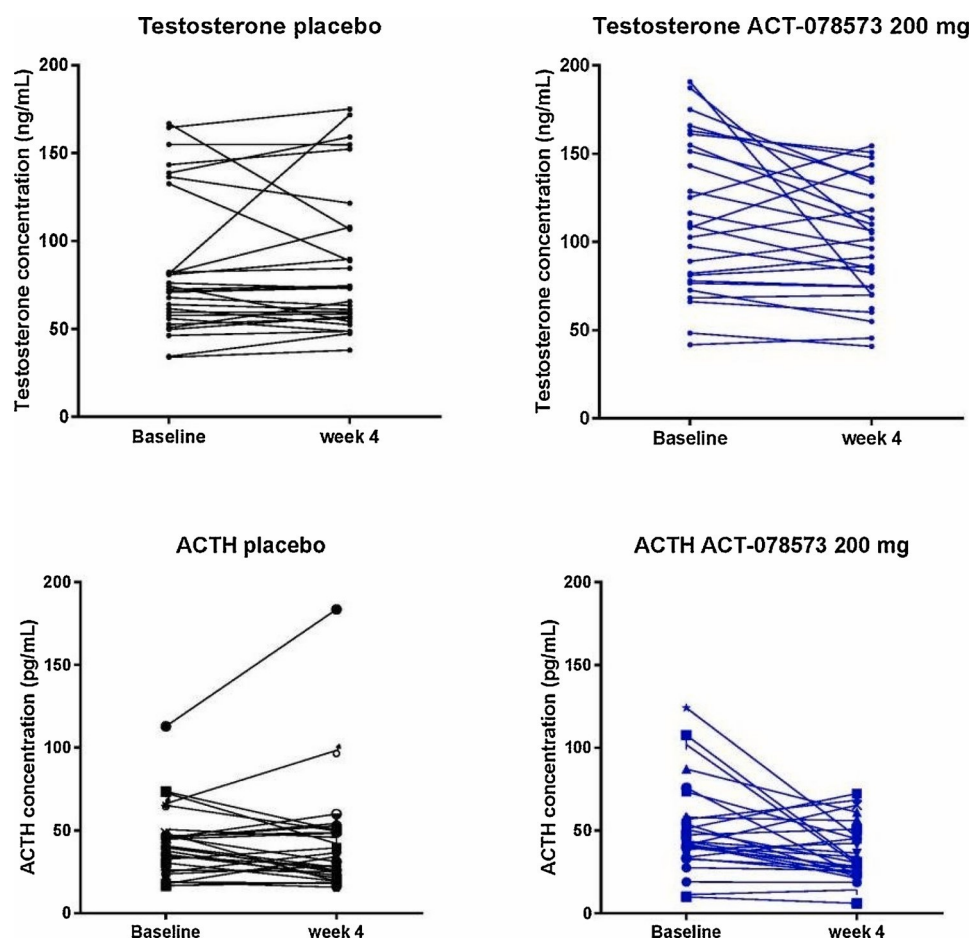


Fig. 5. Individual data points for testosterone and ACTH following 4 weeks of once daily administration of 200 mg almorexant or placebo.

of rat anterior pituitary cells from both male and female animals, no effects of orexin-A and -B were detected on either basal or releasing factor-stimulated secretion of luteinizing hormone, prolactin, TSH or growth hormone [30]. In the present clinical study, plasma or serum levels of DHEA, DHT, TSH, T3, T4, FSH, LH, prolactin, ghrelin, cortisol, inhibin B, and leptin did not change after a 4-week almorexant administration. Testosterone and ACTH plasma concentrations were the only variables shown to be slightly decreased in the almorexant compared to the placebo group (Fig. 5). However, the mean decrease in testosterone concentration was neither reflected in a decrease of DHT, its active metabolite, nor in spermatogenesis variables. Therefore, this decrease was not clinically relevant. Similar to the effect on testosterone levels, also the decrease of ACTH was rated as a chance finding without clinical significance for the following reasons. On the one hand, despite the decrease, ACTH levels remained within the normal range (10–60 pg/mL between 8:00–10:00) and did not affect cortisol levels. Furthermore, the results of a former study [20] in which no treatment-related effects were observed on ACTH plasma concentration-time profiles after almorexant administration of up to 1000 mg once daily for 6 days, confirmed this evaluation. Our data are in agreement with the absence of a clear effect of the orexin system on the hypothalamo-pituitary-gonadal axis [30,45]. In this study hormonal assessments were conducted at fixed time points under standardized conditions and potential effects of almorexant on, e.g., the circadian cycle of ACTH and cortisol were not investigated. However, in the rat it has been demonstrated that almorexant did not affect the normal diurnal release of these hormones [45].

Further, there was no effect of almorexant on the levels of pancreatic elastase in stool. Administration of the selective NPY Y4 ligand pancreatic polypeptide into the rat lateral hypothalamus resulted in the activation of orexinergic neurons [30,46]. However, it is not known whether pancreatic polypeptide is the normal ligand acting at these receptors *in vivo*, also because of uncertainty about the synthesis of this peptide within the brain [30,47,48].

Overall, the safety data collected over 4 weeks of daily almorexant treatment revealed no safety concerns and measures of narcoleptic effects and daytime sleepiness appeared to be unaffected by nighttime almorexant administration. This is in line with the results of other clinical studies with almorexant that have been conducted previously [14,19,20,31,32].

5. Conclusion

In conclusion, none of the toxicity findings that had been found preclinically were observed in this clinical study, suggesting that these preclinical findings are species-specific. The effect and safety profile of a 4-week administration of almorexant 200 mg support the planning and conduct of larger clinical trials that include more human subjects and are of longer duration.

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CRediT authorship contribution statement

Jasper Dingemanse: Writing - original draft. **Pascal Charef:** Writing - review & editing. **Jed Black:** Conceptualization. **Chris Gouws:** Writing - review & editing.

Declaration of Competing Interest

At the time of the study Jasper Dingemanse and Pascal Charef were full-time employees of Actelion Pharmaceuticals Ltd, the predecessor company of Idorsia Pharmaceuticals Ltd.

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References

- [1] L. de Lecea, T.S. Kilduff, C. Peyron, X.-B. Gao, P.E. Foye, P.E. Danielson, C. Fukuhara, E.L.F. Battenberg, V.T. Gautvik, F.S. Bartlett II, W.N. Frankel, A. N. van den Pol, F.E. Bloom, K.M. Gautvik, J.G. Sutcliffe, The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity, *Proc. Natl. Acad. Sci. U.S.A.* 95 (1) (1998) 322–327, <https://doi.org/10.1073/pnas.95.1.322>.
- [2] T.S. Kilduff, C. Peyron, The hypocretin/orexin ligand-receptor system: implications for sleep and sleep disorders, *Trends Neurosci.* 23 (8) (2000) 359–365, [https://doi.org/10.1016/S0166-2236\(00\)01594-0](https://doi.org/10.1016/S0166-2236(00)01594-0).
- [3] T. Sakurai, The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness, *Nat. Rev. Neurosci.* 8 (3) (2007) 171–181, <https://doi.org/10.1038/nrn2092>.
- [4] M.E. Carter, J. Schaich Borg, L. de Lecea, The brain hypocretins and their receptors: mediators of allostatic arousal, *Curr. Opin. Pharmacol.* 9 (1) (2009) 39–45, <https://doi.org/10.1016/j.coph.2008.12.018>.
- [5] N. Tsujino, T. Sakurai, Orexin/hypocretin: a neuropeptide at the interface of sleep, energy homeostasis, and reward system, *Pharmacol. Rev.* 61 (2) (2009) 162–176, <https://doi.org/10.1124/pr.109.001321>.
- [6] T. Sakurai, A. Amemiya, M. Ishii, I. Matsuzaki, R.M. Chemelli, H. Tanaka, S. C. Williams, J.A. Richardson, G.P. Kozlowski, S. Wilson, J.R. Arch, R. E. Buckingham, A.C. Haynes, S.A. Carr, R.S. Annan, D.E. McNulty, W.S. Liu, J. A. Terrett, N.A. Elshourbagy, D.J. Bergsma, M. Yanagisawa, Orexins and orexin receptors : a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior, *Cell.* 92 (4) (1998) 573–585, [https://doi.org/10.1016/S0092-8674\(00\)80949-6](https://doi.org/10.1016/S0092-8674(00)80949-6).
- [7] R. Szymusiak, D. McGinty, Hypothalamic regulation of sleep and arousal, *Ann. NY.*

- J. Biomedicine & Pharmacotherapy* 133 (2021) 110955
- Acad. Sci. 1129 (2008) 275–286, <https://doi.org/10.1196/annals.1417.027>.
- [8] T. Nambu, T. Sakurai, K. Mizukami, Y. Hosoya, M. Yanagisawa, K. Goto, Distribution of orexin neurons in the adult rat brain, *Brain Res.* 827 (1–2) (1999) 243–260, [https://doi.org/10.1016/S0006-8993\(99\)01336-0](https://doi.org/10.1016/S0006-8993(99)01336-0).
- [9] C. Peyron, D.K. Tighe, A.N. van den Pol, L. de Lecea, H.C. Heller, J.G. Sutcliffe, T. S. Kilduff, Neurons containing hypocretin (orexin) project to multiple neuronal systems, *J. Neurosci.* 18 (23) (1998) 9996–10015, <https://doi.org/10.1523/JNEUROSCI.18-23-09996.1998>.
- [10] S.V. Mahler, D.E. Moorman, R.J. Smith, M.H. James, G. Aston-Jones, Motivational activation: a unifying hypothesis of orexin/hypocretin function, *Nat. Neurosci.* 17 (10) (2014) 1298–1303, <https://doi.org/10.1038/nn.3810>.
- [11] S. Nishino, B. Ripley, S. Overeem, G.J. Lammers, E. Mignot, Hypocretin (orexin) deficiency in human narcolepsy, *Lancet* 355 (9197) (2000) 39–40, [https://doi.org/10.1016/S0140-6736\(99\)05582-8](https://doi.org/10.1016/S0140-6736(99)05582-8).
- [12] W.K. Samson, S.L. Bagley, A.V. Ferguson, M.M. White, Orexin receptor subtype activation and locomotor behaviour in the rat, *Acta Physiol. Oxf. (Oxf)* 198 (3) (2010) 313–324, <https://doi.org/10.1111/j.1748-1716.2009.02056.x>.
- [13] S.S. Sullivan, C. Guilleminault, Emerging drugs for insomnia: new frontiers for old and novel targets, *Expert Opin. Emerg. Drugs* 14 (3) (2009) 411–422, <https://doi.org/10.1517/14728210903171948>.
- [14] P. Hoever, G. Dorffner, H. Benes, T. Penzel, H. Danker-Hopfe, M.J. Barbanoj, G. Pillar, B. Saletu, O. Polo, D. Kunz, J. Zeitlhofer, S. Berg, M. Partinen, C. L. Bassetti, B. Hogl, I.O. Ebrahim, E. Holsboer-Trachsler, H. Bengtsson, Y. Peker, U.-M. Hemminger, E. Chiossi, G. Hajak, J. Dingemans, Orexin receptor antagonism, a new sleep-enabling paradigm: a proof-of-concept clinical trial, *Clin. Pharmacol. Ther.* 91 (6) (2012) 975–985, <https://doi.org/10.1038/clpt.2011.370>.
- [15] A. Palasz, D. Lapray, C. Peyron, E. Rojczyk-Gołębiewska, R. Skowronek, G. Markowski, B. Czajkowska, M. Krzystanek, R. Wiaderkiewicz, Dual orexin receptor antagonists - promising agents in the treatment of sleep disorders, *Int. J. Neuropsychopharmacol.* 17 (1) (2014) 157–168, <https://doi.org/10.1017/S1461145713000552>.
- [16] R. Suchting, J.H. Yoon, G.G.S. Miguel, C.E. Green, M.F. Weaver, J.N. Vincent, G. R. Fries, J.M. Schmitz, S.D. Lane, Preliminary examination of the orexin system on relapse-related factors in cocaine use disorder, *Brain Res.* (2020), <https://doi.org/10.1016/j.brainres.2019.146359>.
- [17] M.H. James, J.E. Fragale, R.N. Aurora, N.A. Cooperman, D.D. Langleben, G. Aston-Jones, Repurposing the dual orexin receptor antagonist suvorexant for the treatment of opioid use disorder: why sleep on this any longer? *Neuropsychopharmacology* 45 (5) (2020) 717–719, <https://doi.org/10.1038/s41386-020-0619-x>.
- [18] C. Brisbare-Roch, J. Dingemans, R. Koberstein, P. Hoever, H. Aissaoui, S. Flores, C. Mueller, O. Naylor, J. van Gerven, S.L. de Haas, P. Hess, C. Qiu, S. Buchmann, M. Scherz, T. Weller, W. Fischli, M. Clozel, F. Jenck, Promotion of sleep by targeting the orexin system in rats, dogs and humans, *Nat. Med.* 13 (2) (2007) 150–155, <https://doi.org/10.1038/nm1544>.
- [19] P. Hoever, S. de Haas, J. Winkler, R.C. Schoemaker, E. Chiossi, J. van Gerven, J. Dingemans, Orexin receptor antagonism, a new sleep-promoting paradigm: an ascending single-dose study with almorexant, *Clin. Pharmacol. Ther.* 87 (5) (2010) 593–600, <https://doi.org/10.1038/clpt.2010.19>.
- [20] P. Hoever, S.L. de Haas, G. Dorffner, E. Chiossi, J. van Gerven, J. Dingemans, Orexin receptor antagonism: an ascending multiple-dose study with almorexant, *J. Psychopharmacol. (Oxford)* 26 (8) (2012) 1071–1080, <https://doi.org/10.1177/0269881112448946>.
- [21] M. Hoch, P. Hoever, R. Theodor, J. Dingemans, Almorexant effects on CYP3A4 activity studied by its simultaneous and time-separated administration with simvastatin and atorvastatin, *Eur. J. Clin. Pharmacol.* 69 (6) (2013) 1235–1245, <https://doi.org/10.1007/s00228-012-1470-8>.
- [22] T.C. Neylan, A. Richards, T.J. Metzler, L.M. Ruoff, J. Varbel, A. O'Donovan, M. Sivasubramanian, T. Motraghi, J. Hlavin, S.L. Batki, S.S. Inslicht, K. Samuelson, S.R. Morairty, T.S. Kilduff, Acute cognitive effects of the hypocretin receptor antagonist almorexant relative to zolpidem and placebo: a randomized clinical trial, *Sleep* (2020), <https://doi.org/10.1093/sleep/zsaa080>. Apr 18 Online ahead of print.
- [23] L.A. Grafe, S. Bhatnagar, Orexins and stress, *Front. Neuroendocrinol.* 51 (2018) 132–145, <https://doi.org/10.1016/j.yfrne.2018.06.003>.
- [24] M.H. James, E.J. Campbell, C.V. Dayas, Role of the orexin/hypocretin system in stress-related psychiatric disorders, *Curr. Top. Behav. Neurosci.* 33 (2017) 197–219, https://doi.org/10.1007/7854_2016_56.
- [25] World Health Organization, WHO Laboratory Manual for the Examination and Processing of Human Semen, fifth edition, 2010.
- [26] C. Sturzenegger, C.L. Bassetti, The clinical spectrum of narcolepsy with cataplexy: a reappraisal, *J. Sleep Res.* 13 (2004) 395–406, <https://doi.org/10.1111/j.1365-2869.2004.00422.x>.
- [27] M.W. Johns, A new method for measuring daytime sleepiness: the Epworth sleepiness scale, *Sleep.* 14 (6) (1991) 540–545, <https://doi.org/10.1093/sleep/14.6.540>.
- [28] M.H. Ring, D.F. Rabenstein, J. Horwath-Winter, I. Boldin, R. Horantner, T. Haslwanter, Introducing a new parameter for the assessment of the tear film lipid layer, *Invest. Ophthalmol. Vis. Sci.* 53 (2012) 6638–6644, <https://doi.org/10.1167/iovs.12-10257>.
- [29] R.M. Schiffman, M.D. Christianson, G. Jacobsen, J.D. Hirsch, B.L. Reis, Reliability and validity of the ocular surface disease index, *Arch. Ophthalmol. (Paris)* 118 (5) (2000) 615–621, <https://doi.org/10.1001/archophth.118.5.615>.
- [30] A.V. Ferguson, W.K. Samson, The orexin/hypocretin system: a critical regulator of neuroendocrine and autonomic function, *Front. Neuroendocrinol.* 24 (3) (2003) 141–150, [https://doi.org/10.1016/S0091-3022\(03\)00028-1](https://doi.org/10.1016/S0091-3022(03)00028-1).
- [31] J. Black, G. Pillar, J. Hedner, Efficacy and safety of almorexant in adult chronic insomnia: a randomized placebo-controlled trial with an active reference, *Sleep Med.* 36 (2017) 89–94, <https://doi.org/10.1016/j.sleep.2017.05.009>.
- [32] T. Roth, J. Black, R. Cluydts, R. Cluydts, P. Charef, M. Cavallaro, F. Kramer, G. Zammit, J. Walsh, Dual orexin receptor antagonist, almorexant, in elderly patients with primary insomnia: a randomized, controlled study, *Sleep* 40 (2) (2017) 1–8, <https://doi.org/10.1093/sleep/zsw034>.
- [33] R. Rosenzweig, N. Miget, S. Brohler, Transaminase elevation on placebo during Phase I trials: prevalence and significance, *Br. J. Clin. Pharmacol.* 48 (1) (1999) 19–23, <https://doi.org/10.1046/j.1365-2125.1999.00952.x>.
- [34] M. Senchyna, M.B. Wax, Quantitative assessment of tear production: a review of methods and utility in dry eye drug discovery, *J. Ocul. Biol. Dis. Infor.* 1 (1) (2008) 1–6, <https://doi.org/10.1016/j.psyneuen.2012.07.016>.
- [35] E. Knop, N. Knop, T. Millar, The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland, *Invest. Ophthalmol. Vis. Sci.* 52 (4) (2011) 1938–1978, <https://doi.org/10.1167/iovs.10-6997c>.
- [36] A.J. Thody, S. Shuster, Control and function of sebaceous glands, *Physiol. Rev.* 69 (1989) 383–416, <https://doi.org/10.1152/physrev.1989.69.2.383>.
- [37] S.M. Cox, J.J. Nichols, The neurobiology of the meibomian glands, *Ocul. Surf.* 12 (3) (2014) 167–177, <https://doi.org/10.1016/j.jtos.2014.01.005>.
- [38] N. Yokoi, F. Mossa, J.M. Tiffany, A.J. Bron, Assessment of meibomian gland function in dry eye using meibometry, *Arch. Ophthalmol.* 117 (6) (1999) 723–729, <https://doi.org/10.1001/archophth.117.6.723>.
- [39] P. Versura, G. Giannaccare, E.C. Campos, Sex-steroid imbalance in females and dry eye, *Curr. Eye Res.* 40 (2) (2015) 162–175, <https://doi.org/10.3109/02713683.2014.966847>.
- [40] D.A. Sullivan, H. Yamagami, M. Liu, R.J. Steagall, F. Schirra, T. Suzuki, K. L. Krenzer, J.M. Cermak, R.M. Sullivan, S.M. Richards, D.A. Schaumberg, M. R. Dana, B.D. Sullivan, Sex steroids, the meibomian gland and evaporative dry eye, *Adv. Exp. Med. Biol.* 506 (Part A) (2002) 389–399, https://doi.org/10.1007/978-1-4615-0717-8_56.
- [41] P. Cho, M. Yap, Schirmer test. I. A review, *Optom. Vis. Sci.* 70 (2) (1993) 152–156, <https://doi.org/10.1097/00006324-199302000-00011>.
- [42] P. Cho, M. Yap, Schirmer test. II. A clinical study of its repeatability, *Optom. Vis. Sci.* 70 (2) (1993) 157–159, <https://doi.org/10.1097/00006324-199302000-00012>.
- [43] R.P. Amann, The cycle of the seminiferous epithelium in humans: a need to revisit? *J. Androl.* 29 (5) (2008) 469–487, <https://doi.org/10.2164/jandrol.107.004655>.

- J. *Dingemanse*
Biomedicine & Pharmacotherapy 133 (2021) 110955
- [44] J.P. McCulley, W.E. Shine, The lipid layer of tears: dependent on meibomian gland function, *Exp. Eye Res.* 78 (3) (2004) 361–365, [https://doi.org/10.1016/s0014-4835\(03\)00203-3](https://doi.org/10.1016/s0014-4835(03)00203-3).
- [45] M.A. Steiner, C. Sciarretta, C. Brisbare-Roch, D.S. Strasser, R. Studer, F. Jenck, Examining the role of endogenous orexins in hypothalamus-pituitary-adrenal axis endocrine function using transient dual orexin receptor antagonism in the rat, *Psychoneuroendocrinology*. 38 (4) (2012) 560–571, <https://doi.org/10.1016/j.psyneuen.2012.07.016>.
- [46] R.E. Campbell, M.S. Smith, S.E. Allen, B.E. Grayson, Jarlath M.H. French-Mullen, Kevin L. Grove, Orexin neurons express a functional pancreatic polypeptide Y4 receptor, *J. Neurosci.* 23 (4) (2003) 1487–1497, <https://doi.org/10.1523/JNEUROSCI.23-04-01487.2003>, 15.
- [47] K. Miyazaki, A. Funakoshi, Distribution of pancreatic polypeptide-like immunoreactivity in rat tissues, *Regul. Pept.* 21 (1-2) (1988) 37–43, [https://doi.org/10.1016/0167-0115\(88\)90089-4](https://doi.org/10.1016/0167-0115(88)90089-4).
- [48] V.A. Pieribone, L. Brodin, K. Friberg, J. Dahlstrand, C. Soderberg, D. DLarhammar, T. Hokfelt, Differential expression of mRNAs for neuropeptide Y-related peptides in rat nervous system tissues: possible evolutionary conservation, *J. Neurosci.* 12 (9) (1992) 3361–3371, <https://doi.org/10.1523/JNEUROSCI.12-09-03361.1992>.

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